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Apoptosis

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13. ABSTRACT (Maximum 200 Words) We have recently observed that certain sphingolipids (e.g., dimethyl-sphingosine) induce apoptosis in vitro in tumor cells despite the over-expression of HER-2/neu, P-gp-170 and other resistance mechanisms relevant to breast cancer. The purpose of the current studies was to translate the formulation and toxicity studies of liposomal-dimethyl-sphingosine (L-DMSP) conducted in the previous years to initial proof-of-principle studies in the nude mouse/human HER-2/neu over-expressing MDA-MD-361 breast adenocarcinoma model. Mice were treated with a multiple-dose MTD regimen of L-DMSP (4.5 mg DMSP per injection, i.v.) beginning either one-week after tumor implantation or when tumors grew to 4-5 mm diameter. Early treatment caused a delay in or reduced subsequent tumor growth, but was rarely curative. The tumor growth curve was suggestive of stasis, with anti-tumor effects evident for as long as ~30 days after cessation of treatment. Treatment initiated at the later timepoint was also efficacious, but less so than with early treatment; the response appeared to be primarily very brief stasis, with slight growth occurring through treatment, followed by a slower growth rate than for controls. Future studies will be directed to optimization of the L-DMSP formulation, dose and schedule, as well as to the definition of the mechanism of its anti-tumor activity.					
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Introduction

Over-expression of HER-2/neu has been linked to poorer prognosis and reduced survival in breast cancer patients. The basis for this association is likely multifactorial and includes therapeutic resistance, such as resistance to Taxol (paclitaxel), widely used in many chemotherapeutic regimens for this disease. We have recently observed that certain sphingolipids (e.g., dimethyl-sphingosine), either as free lipids or as constituents of liposomes, induce apoptosis *in vitro* in tumor cells despite the over-expression of HER-2/neu, P-gp-170 and other resistance mechanisms relevant to breast cancer.

The purpose of the current studies was to translate the formulation and toxicity studies of liposomal-dimethyl-sphingosine (L-DMSP) conducted in the previous years to initial proof-of-principle studies in nude mouse/human HER-2/neu over-expressing breast adenocarcinoma models. Investigations leading to and pertinent to Aims 5 (efficacy studies) were the main focus. We present herein evaluation of the anti-tumor efficacy of L-DMSP in the human HER-2/neu over-expressing breast adenocarcinoma orthotopic xenograft model, MDA-MB-361.

Progress in this Aim has been acceptable and studies are continuing in the non-funded extension year.

Body

Task 4

Initial formulations of long-circulating (PEG-containing) liposomes (PEG-L-DMSP) have been prepared by the lipid film hydration and extrusion methods. The composition was DMSP/DPPC/DSPE, 1:2:2:0.4. No difficulties in preparing this formulation were encountered.

Tasks 5 and 6

In the first year, studies in nude mice indicated that a multiple-dose MTD for DMSP (as L-DMSP) of 4.0 mg was a more accurate figure than the 0.5-1.5 mg previously suggested by the literature and by our preliminary studies. These studies have now been completed and confirm a value of 4.0-4.5 mg per injection for the standard (non-stealth) formulation (DMSP/DPPC/DSPE, 1:2:2) in a multiple-dose regimen.

Task 7

Task 7, using long-circulating (PEG-containing) liposomes will be narrowed to nude mouse studies, foregoing studies in BALB/c mice.

Task 8

SUV liposome formulations of DMSP (L-DMSP) were prepared by lipid film hydration and repeated extrusion techniques. The mole composition was DMSP/DPPC/DSPE, 1:2:2.

MDA-MB-361 human HER-2/neu-over-expressing breast adenocarcinoma cells were obtained from ATCC and cultured in CO₂-free Liebowitz L-15 medium; these specific culture conditions were required to maintain the original cell morphology and tumorigenicity. $4-6 \times 10^6$ cells were implanted in the mammary fat pad of 6-9 week old female nude mice. Mice were treated with a multiple-dose MTD regimen of L-DMSP (4.5 mg DMSP per injection, i.v.) beginning either one-week later or when tumors grew to 4-5 mm diameter. Tumor growth was monitored by caliper measurements.

Early treatment (one week after tumor implantation) with a multiple-dose (five injections over about two weeks) regimen of L-DMSP (4.5 mg DMSP per injection; 20 mole percent of an SUV formulation), caused a delay in or reduced subsequent tumor growth, but was apparently curative in only one of eight mice (Fig. 1 and 2). The tumor growth curve was suggestive of stasis, with anti-tumor effects evident for as long as ~30 days after cessation of treatment; the caveat in this experiment was two toxic deaths that occurred in this group within a week after the last injection. When the five injections were administered over a slightly longer timeframe (16 vs. 14 days) or to mice that were ~4 weeks older, no deaths occurred, and a slower rate of tumor growth than for the controls was still observed.

When administration was initiated at the later timepoint (tumor diameters, 4-5 mm), treatment with L-DMSP was also efficacious, but less so than with early treatment. The effects of late treatment with L-DMSP (Fig. 1) appeared to be primarily very brief stasis, with slight growth occurring through treatment, followed by a slower growth rate than for controls.

Task 9

This Task is currently being undertaken since initial results from Task 8 are available, allowing comparisons of the anti-tumor efficacy of long-circulating, PEG-SUVs to those of the non-targeted SUVs.

Tasks 10 and 11

We have placed these studies in a lower priority than the nude mouse studies, and may not undertake them in light of the emphasis on the mouse models.

Tasks 12 and 13

These studies are certainly still planned, but have taken a lower priority than establishing the HER-2/neu tumor model for evaluation of anti-tumor efficacy. They will be conducted with both non-targeted SUVs and PEG-SUVs.

Task 14

The most critical experiments to repeat will be those supporting the proof-of-principle, anti-tumor efficacy studies.

Key Research Accomplishments

Re-established human MDA-MB-361 HER-2/neu-over-expressing orthotopic human breast adenocarcinoma xenograft model in female nude mice

Identified positive, durable anti-tumor efficacy of a multiple-dose MTD regimen of L-DMSP (conventional SUVs) in the 361 model, with both early (one week post-implantation) and late (tumor diameters, 4-5 mm) treatments

Reportable Outcomes

Two abstracts (Era of Hope Meeting, September, 2002, Orlando, FL, "Liposomal-dimethylsphingosine and paclitaxel copolymer are active against HER-2/neu-overexpressing human breast adenocarcinoma orthotopic xenograft model"; EORTC/AACR/NCI Meeting, November, 2002, Frankfurt, Germany, "Evaluation *in vivo* of new agents for drug-resistant ovarian and

breast carcinomas") were accepted and poster presentations have been given; a manuscript is planned once the next L-DMSP formulations have been evaluated.

Conclusions

We conclude that the sphingolipid, DMSP, administered as a liposomal (SUV) formulation, has anti-tumor efficacy against the HER-2/neu over-expressing MDA-MB-361 human breast adenocarcinoma orthotopic nude mouse xenograft model, evident with either lower or higher tumor burden. Future studies will be directed to optimization of the L-DMSP formulation, dose and schedule, as well as to the definition of the mechanism of its anti-tumor activity.

References

- Endo K, Igarashi Y, Nisar M, Zhou Q and Hakamori S-I. Cell membrane signaling as target in cancer therapy: inhibitory effect of N, N-dimethyl and N, N, N-trimethyl sphingosine derivatives on *in vitro* and *in vivo* growth of human tumor cells in nude mice. *Cancer Res.* 51: 1613-1618, 1991.
- Okoshi H, Hakamori S-I, Nisar M, Zhou Q, Kimura S, Tashiro K and Igarashi Y. Cell membrane signaling as target in cancer therapy II: Inhibitory effect of N, N, N-trimethylsphingosine on metastatic potential of murine B16 melanoma cell line through blocking of tumor cell-dependent platelet aggregation. *Cancer Res.* 51: 6019-6024, 1991.
- Park YS, Hakamori S-I, Kawa S, Ruan F and Igarashi Y. Liposomal N, N, N-trimethylsphingosine (TMS) as an inhibitor of B16 melanoma cell growth and metastasis with reduced toxicity and enhanced drug efficacy compared to free TMS: cell membrane signaling as a target in cancer therapy III. *Cancer Res.* 54: 2213-2217, 1994.
- Sweeney EA, Sakakura C, Shirahama T, Masamune A, Ohta H, Hakamori S-I and Igarashi Y. Sphingosine and its methylated derivative N, N-dimethylsphingosine (DMS) induce apoptosis in a variety of human cancer cell lines. *Int. J. Cancer* 66: 358-366, 1996.
- Shirahama T, Sweeney EA, Sakakura C, Singhal AK, Nishiyama S-i, Hakomori S-i and Igarashi Y. *In vitro* and *in vivo* induction of apoptosis by sphingosine and N,N-dimethylsphingosine in human epidermoid carcinoma KB-3-1 and its multidrug-resistant cells. *Clin. Cancer Res.* 3: 257-264, 1997.

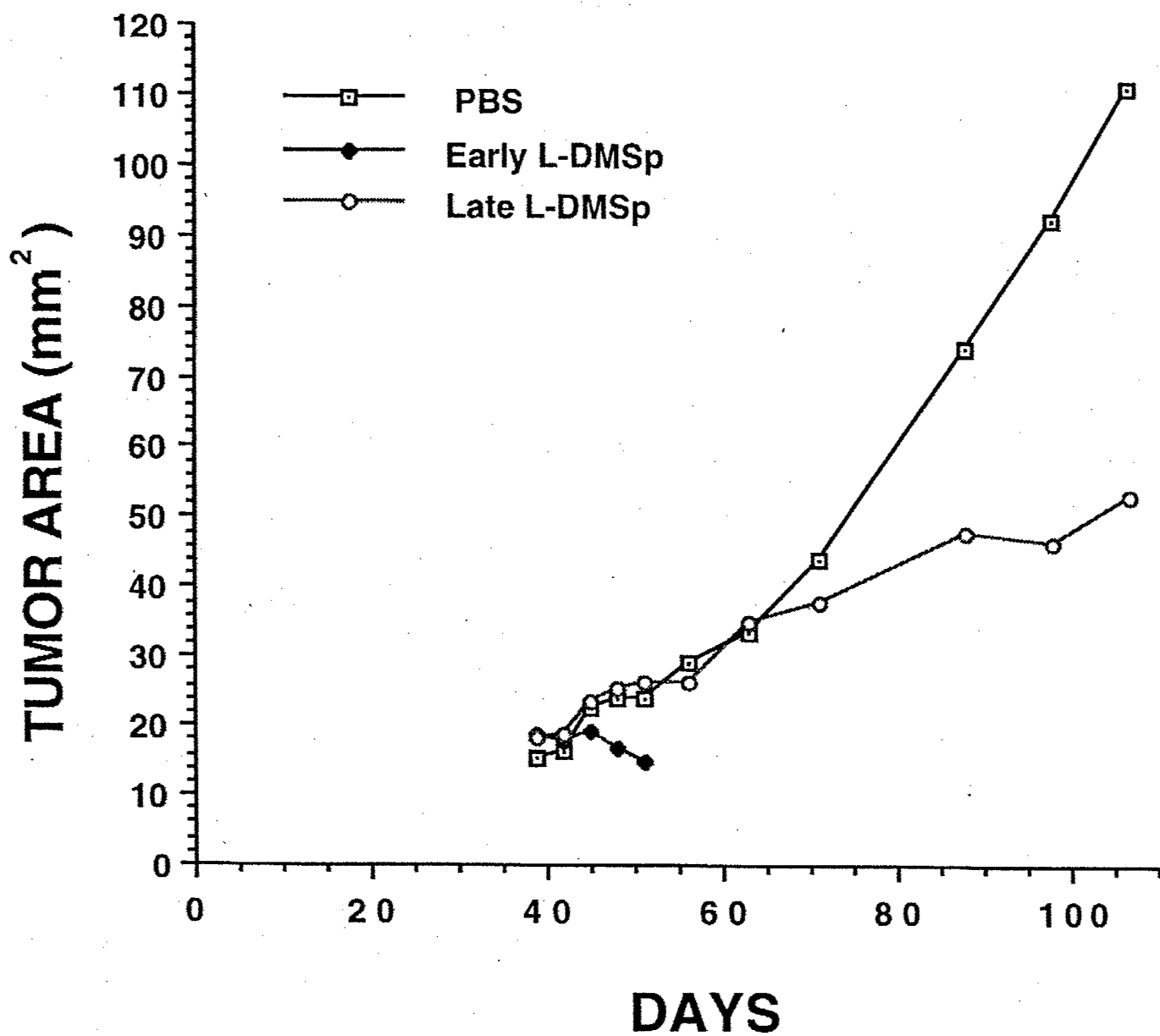


Figure 1

Responses of 361 model to L-DMSp

L-DMSp was composed of DMSP/DPPC/DSPC. Formulation was administered i.v. on Days 7, 10, 14, 18 and 21 (early treatment), or on Days 38, 42, 45, 48 and 51 (late treatment). Two deaths from drug toxicity occurred in the early treatment group on Days 23 and 28.

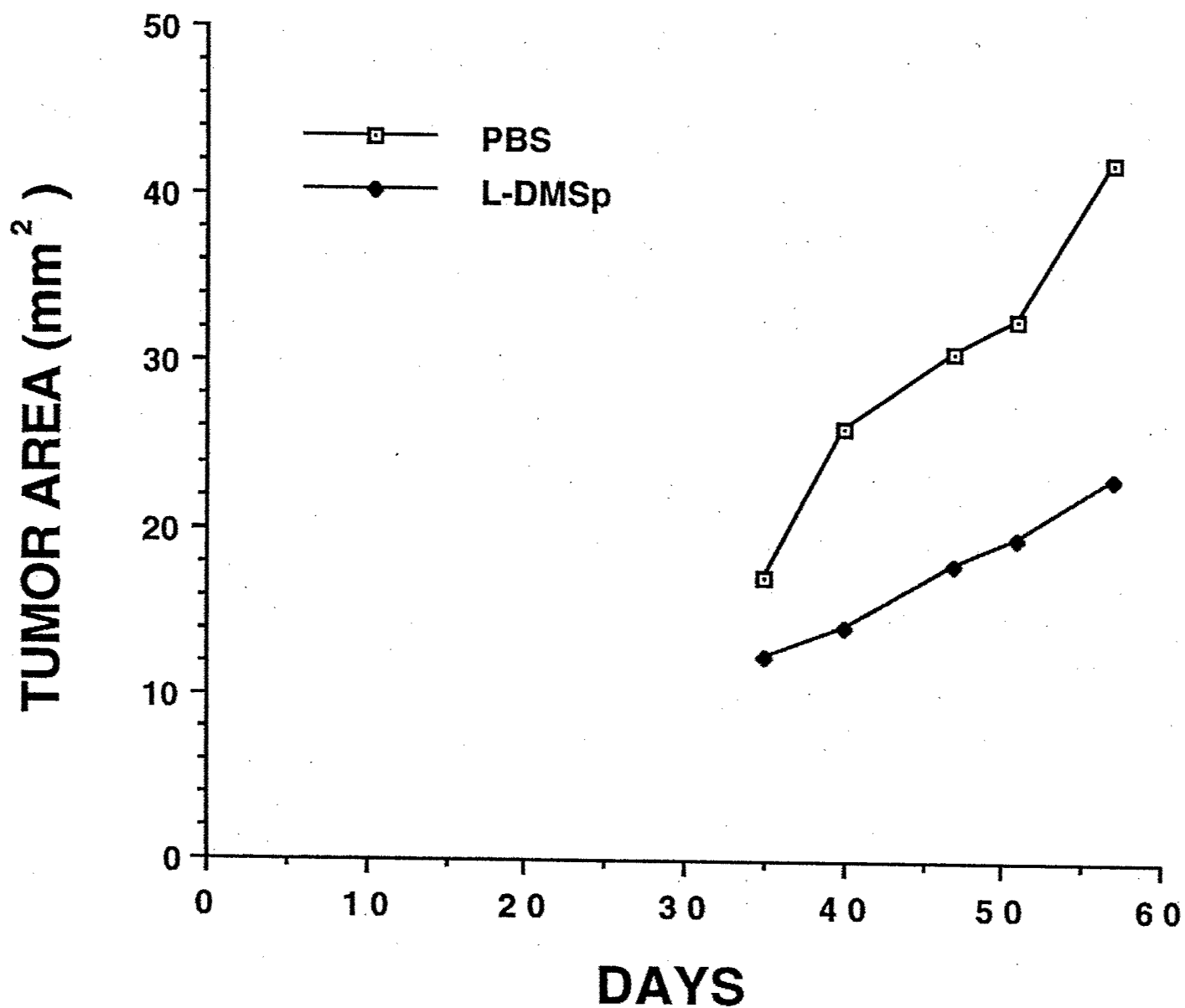


Figure 2

Responses of 361 model to L-DMSP
L-DMSP was composed of DMSP/DPPC/DSPC. Formulation was administered i.v. on Days 7, 12, 16, 19 and 23. No deaths from drug toxicity occurred.